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Discovery of novel aminothiadiazole amides as selective EP_3 receptor antagonists

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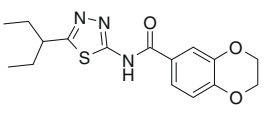
ABSTRACT

This Letter discloses a series of 2-aminothiadiazole amides as selective EP_3 receptor antagonists. SAR optimization resulted in compounds with excellent functional activity in vitro. In addition, efforts to optimize DMPK properties in the rat are discussed. These efforts have resulted in the identification of potent, selective EP_3 receptor antagonists with excellent DMPK properties suitable for in vivo studies.

The EP_3 receptor is a 7-transmembrane (7-TM) G-protein coupled receptor found in various human tissues including the kidney,[1] uterus,[2] bladder,[3] stomach,[4] and brain.[5] Prostaglandin E_2 (PGE_2), a primary product of arachidonic acid metabolism by the cyclooxygenase pathway, is the natural ligand attributed to agonism of EP_3 as well as other EP receptor subtypes, EP_{1-4}.[6] Specifically, EP_3 receptor activity has been implicated in uterine contraction,[7] gastric acid secretion,[8] fever mediation,[9] bladder contraction,[10] and smooth muscle contraction of the GI tract. Past efforts to elucidate the physiological role of EP_3 have utilized agonists such as PGE_2 and other close analogs.[11] Since PGE_2 retains agonist activity at all four EP receptors, an alternative ligand with good receptor subtype selectivity is needed to identify the specific physiological role of EP_3. Recently, more selective inhibitors have been disclosed.[12]

In the course of our investigation to identify new selective antagonists, aminothiadiazole **1** was identified from a high-throughput screen as having good antagonist activity for human EP_3 (Fig. 1).[13] In addition, **1** demonstrated excellent selectivity against other EP subtypes as well as the DP, FP, TP, and IP prostenoid receptors. Despite having a short half-life and low oral bioavailability in the rat, the in vitro profile for **1** was an excellent starting point for lead optimization.

Initial optimization efforts were focused on substitution of the five-position of the thiadiazole ring (Table 1). Interestingly, while an unsubstituted phenyl group resulted in a loss of activity (**4**) relative to alkyl substitution (**1**–**3**), substitution on the phenyl ring had a pronounced affect on activity (**5–11**). Substitution at both



1

human EP_3 (Ki) = 100 nM
EP_1, EP_4, TP, FP, IP, DP (Ki) = All > 25 μM

Rat PK

$T_{1/2}$ (h) = 0.38
Cl (mL/min/kg) = 110
Oral F (%) = 20

Figure 1. High-throughput screening hit **1**.

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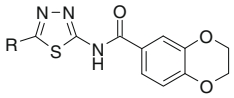
Table 1
In vitro functional data and rat DMPK properties for 5-substituted thiadiazoles[13]



Compound	R	FLIPR hEP$_3$ fpK_i[a]	Rat PK.[b]		
			Cl (mL/min/kg)	*T*½ (h)	Oral F (%)
1	3-Pentyl	7.1	110	0.38	20
2	Cyclopentyl	6.7	88	1.1	14
3	*tert*-Butyl	6.8	290	0.23	11
4	Ph	<4.6			
5	4-Me–Ph	<4.6			
6	2-Me–Ph	6.3	53	1.3	38
7	2,6-Di-Me–Ph	7.9	29	0.91	51
8	2,4,6-Tri-Me–Ph	7.7	2.9	9.8	65
9	2,3,5,6-Me–Ph	6.4			
10	2,6-Di-Cl–Ph	8.5	17	2.6	100
11	2,6-Di-Cl-4-MeO–Ph	7.9	3.7	2.2	98

[a] Values are a mean of at least two determinations with a SEM < ±0.1 log units.
[b] DMPK properties are averaged values (n = 3) from an oral/iv po study in Sprague–Dawley rats dosed at 2 mg/kg (oral) and 1 mg/kg (iv).

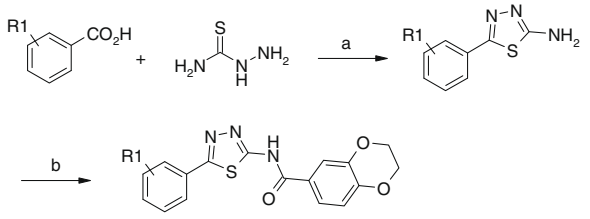
ortho-positions was critical for achieving good functional activity, with halogen and alkyl groups being optimal (**7** and **10**). Substitution at the *para*-position was well tolerated (**8** and **11**) while *meta*-substitution proved detrimental to functional activity (**9**). Incorporating an aromatic group at the five-position of the thiadiazole ring also resulted in enhanced rat DMPK properties relative to simple alkyl substitution at this position. In general, the 5-arylthiadiazoles had substantially lower clearance and longer half-lives than the 5-alkylthiadiazole analogs with an increase in oral bioavailability. In addition to improved functional activity, successive *ortho*- and *para*-substitution resulted in significant decreases in metabolic clearance as exemplified in methylated compounds **6**–**8**.

Compounds in this series were synthesized via precedented procedures starting with the condensation of semicarbathiazide with the desired carboxylic acid to provide the 5-aryl-2-aminothiadiazole (Scheme 1).[14] The aminothiadiazole was converted to the corresponding amide through a BOP-mediated coupling with the requisite carboxylic acid.

Further SAR optimization of the amide portion of the molecule was conducted using *ortho*-disubstituted phenyl groups in the 5-position of the thiadiazole (Table 2). N-Methylation of the amide nitrogen resulted in complete loss of activity indicating that the hydrogen-bond donor properties of the amide are critical to substrate binding with the receptor. A survey of alternative benzoic acids leading to the targeted amides revealed very tight SAR favoring fused heterobicycles as evidenced by the significant decrease in potency for 3,4-dimethoxybenzamide **12**. Both ring contraction and ring expansion of the fused dioxane ring resulted in decreased potency. As a result, optimization efforts were focused on other fused six-membered ring analogs.

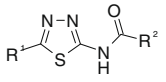
To this end, the fused dioxane was replaced with a fused morpholine residue (Table 3).[15] This modification resulted in an increase in potency as evidenced by benzoxazine **18** compared to compound **7**. Interestingly, incorporation of either exocyclic or endocyclic carbonyl groups exemplified by acetate **20** and benzoxazinone **21**, respectively, resulted in a substantial loss in activity. This suggests that the carbonyl groups in **20** and **21** engage in unfavorable steric or electronic interactions with the receptor, and the proton acceptor properties of the benzoxazine nitrogen may partake in beneficial binding with the receptor substrate providing a modest improvement in ligand potency.

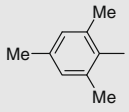
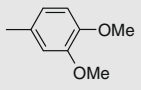
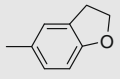
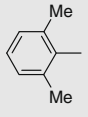
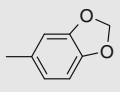
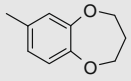
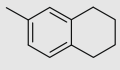
Upon completing our survey of both the amide group and the 5-arylthiadiazole region, optimized combinations were evaluated in an effort to maximize potency and to explore selectivity and rat DMPK properties. With this goal in mind, benzoxazine carboxylic



Scheme 1. Synthesis of 5-aryl-2-aminothiadiazole amides. Reagents and conditions: (a) $POCl_3$, 60 °C, 18 h; (b) BOP, (^{i}Pr)$_2$NEt, DMF, 25 °C.

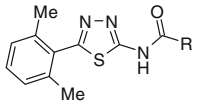
Table 2
Amide group SAR

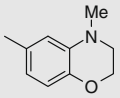
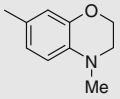
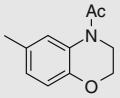
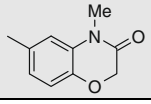


Compound	R1	R2	FLIPR hEP$_3$ fpK_i[a]
12			6.2
13			5.9
14			6.7
15			7.0
16			6.7
17			7.6

[a] Values are a mean of at least two determinations with a SEM < ±0.1 log units.

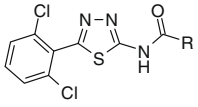
Table 3
Human EP_3 activity across benzoxazine SAR

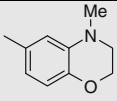
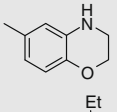
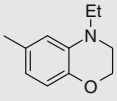


Compound	R	FLIPR hEP$_3$ fpK_i[a]
18		8.3
19		7.8
20		6.1
21		5.7

[a] Values are a mean of at least two determinations with a SEM < ±0.1 log units.

Table 4
In vitro activity and rat DMPK properties



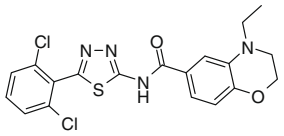
Compound	R	FLIPR hEP$_3$ fpK_i[a]	Rat DMPK properties[b]		
			*T*½ (h)	Cl (mL/min/kg)	Oral F (%)
22		8.9	0.23	22	97
23		8.1	1.1	41	70
24		8.9	5.2	6.5	100

[a] Values are a mean of at least two determinations with a SEM < ±0.1 log units.
[b] DMPK properties are averaged values ($n = 3$) from an oral/iv po study in Sprague–Dawley rats dosed at 2 mg/kg (oral) and 1 mg/kg (iv).

acids were coupled to 2-amino-5-(2,6-dichlorophenyl)thiadiazole (Table 4). Although a high level of potency was achieved in vitro, *N*-methylbenzoxazine amide **22** demonstrated high clearance resulting in a short half-life in the rat. The exceptional oral bioavailability and potency prompted investigation into the nature of the metabolic clearance. We hypothesized that the high clearance might be attributed to *N*-methyl dealkylation. To test this hypothesis the unsubstituted benzoxazine **23** and *N*-ethylbenzoxazine **24** were synthesized. Analysis of **23** and **24** indicates that N-alkylation mitigates benzoxazine metabolism with *N*-ethylamine **24** having a dramatically diminished rate of clearance with a significantly longer half-life.

Having identified compound **24** as a potent antagonist against human EP_3 with excellent rat PK properties, we evaluated its potency at rat and dog EP_3 receptors as well as its selectivity against other prostenoid receptors (Table 5). Additionally, since prostenoid synthesis is dependant upon the oxidative metabolism of arachidonic acid, COX1/2 activity was assayed. Activity at the rat and dog receptors was comparable to that for the human receptor with similar selectivity against the rat EP_1 receptor. In addition, no agonist activity was observed for compounds in this series at the human EP_3 receptor as exemplified by compound **24**.

Table 5
In vitro selectivity and orthologue activity data for **24**[16]



Selectivity (pK_i)	EP_1 <4.6	EP_2 <5.0	EP_4 <5.0	DP <5.0	FP <4.6
TP <5.0	Cox 1 <4.6	Cox 2 <4.6	rat-EP_3 8.2	dog-EP_3 7.9	h-EP_3 pEC_{50} <4.6

In conclusion, optimization of the 2-aminothiadiazole template has yielded potent EP_3 antagonists with excellent rat PK properties and broad cross-species activity as exemplified by compound **24**. In addition, this compound class shows excellent selectivity against other prostenoid receptors including other EP subtypes making this series a valuable tool for identifying and validating potential therapeutic benefits resulting from selective EP_3 inhibition.

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16. Selectivity screening for compound **24** was conducted using radioligand binding assays for the hEP1, hEP2, hEP4, and hTP receptors as detailed in Ref. 9. Binding studies for the hFP receptor were completed by Cerep (Redmond, WA). COX1 and COX2 inhibition was measured using human whole blood in ELISA assay format as described in detail in the following references: (a) Warner, T. D.; Giuliano, F.; Vojnovic, I.; Bukasa, A.; Mitchell, J. A.; Vane, J. R. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 7563; (b) Patrignani, P.; Panara, M. R.; Greco, A.; Fusco, O.; Natoli, C.; Iacobelli, S.; Cipollone, F.; Ganci, A.; Creminon, C.; Maclouf, J.; Patrono, C. *J. Pharmacol. Exp. Ther.* **1994**, *271*, 1705.